leukemic cells (Reynaud et al., 2011). Could this cytokine also transform the function of the normal HSPCs? Consistent with the different effects of the leukemic cells on normal HSCs and progenitors, expression of the IL-6 receptor was found to be much higher in the latter. The authors went on to perform loss-of-function experiments using IL-6 blocking antibodies and an inducible Mx1-Cre;IL-6R$\alpha^{flox/flox}$ system. Both the pharmacological blockade and genetic deletion experiments showed a complete yet transient phenotypic reversion in leukemic-exposed hematopoietic progenitors. Reduced IL-6 production and rescue of the induced leukemic-like phenotypes were also observed in chimeric mice treated with imatinib, the tyrosine-kinase inhibitor currently used to treat CML patients.

To address the relevance to human disease, Welner et al. (2015) cultured adult bone marrow CD34$^+$ cells mixed with bone marrow from either healthy subjects or CML patients, obtaining similar results as in the mouse model, i.e., increased IL-6 content and CD34$^+$ cell proliferation, but decreased lymphoid production. These phenotypes were also reverted by IL-6 inhibition.

A recent study has shown that both the mutated and non-mutated leukemic-exposed hematopoietic cells produce inflammatory cytokines in MPN (Kleppe et al., 2015). This study found IL-6 to be mainly secreted by the mutated cells. Although the mutations explored in the Welner et al. (2015) study and the Kleppe et al. (2015) study are different, parallels from both studies point toward a scenario in which IL-6 secretion by mutant cells transforms normal hematopoietic cells into leukemic-like cells.

In summary, several alterations of normal bone marrow cells induced by mutated HSPCs are required for the manifestation and progression of MPN (Figure 1): (1) neuroglial damage in the bone marrow and induced apoptosis of HSC-niche forming nestin$^+$ MSC, leading to uncontrolled proliferation of mutated HSCs (Arranz et al., 2014); (2) production of pro-inflammatory cytokines by leukemic and normal cells (Reynaud et al., 2011; Kleppe et al., 2015); (3) induction of some leukemic-like features in the normal hematopoietic progenitors (Welner et al., 2015); and (4) excessive proliferation of osteoblastic precursors that become less supportive for hematopoietic progenitors (Krause et al., 2013; Schepers et al., 2013).

Targeting the non-mutated bone marrow cells therefore opens up an attractive door for the development of new therapeutic strategies.

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Defining the Molecular Landscape of Ependymomas

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Ependymomas have a variable prognosis. In this issue of *Cancer Cell*, Pajtler and colleagues identify nine subgroups of ependymoma using DNA methylation profiles. Two subgroups, predominately pediatric, are responsible for most of the mortality, with all others having nearly 100% overall survival after 5 years.

For the past several decades, there has been little progress in the treatment of ependymomas. Currently, treatment is largely restricted to maximal surgical resection and, in some cases, external beam radiation. The tumors do not consistently respond to chemotherapy, and clinical behavior is not accurately predicted by histologic grade. Current

histologic criteria divide the tumors into grade I subependymomas or myxopapillary ependymomas, grade II ependymomas, and grade III anaplastic ependymomas. Grade II ependymomas are the most common ependymomas and are further subdivided into cellular, papillary, clear cell, and tancytic (Korshunov et al., 2010). These subgroupings are imprecise and, to a degree, subjective, limiting their usefulness in treatment decision making.

In this issue of *Cancer Cell*, Pajtler et al. (2015) address these challenges using DNA methylation profiling to test the largest ependymoma cohort studied to date. Their approach is made possible by methods that enabled them to acquire high quality data from formalin fixed paraffin embedded tumor tissue, allowing them to perform DNA methylation profiling on 500 samples from 12 institutions. Technologies to monitor DNA methylation patterns are emerging as an important new approach for cancer classification (Hovestadt et al., 2014). DNA is methylated during development at cell fate decisions to forever alter gene expression in the cell and its daughters. These patterns reflect both cell-of-origin and etiology of the disease. DNA is methylated at cytosine residues and CpG islands by DNA methylation transferase enzymes. DNA methylation marks are inherited and copied through every round of cell division by DNMT1 or can be dynamically modified by so called de novo methyltransfersases such as DNMT3A/B. Hypermethylation leads to inactive heterochromatin and this generally leads to an irreversible repressive state, such as during X chromosome inactivation and differentiation (Robertson, 2005). Because DNA methylation marks are so durable, they can be recovered from small amounts of DNA stored over years in formalin fixed paraffin embedded tissue or in frozen tumor samples with RNA degradation.

Tumor samples from all CNS compartments are examined, including the spinal (SP), posterior fossa (PF), and supratentorial (ST) regions. Using unsupervised hierarchical clustering, they show that tumors in each CNS compartment are comprised of three subgroups, which mostly correspond to histology. Spinal cord tumors included subependymoma (SP-SE), myxopapillary (SP-MPE), and anaplastic ependymoma (SP-EPN). Mutations in the gene linked to neurofibromatosis type 2 (*NF2*) or deletions of the *NF2* locus on chromosome 22q, previously associated with spinal cord ependymomas, were limited to the SP-EPN subgroup.

Posterior fossa ependymoma subgroups include subependymoma (PF-SE) and previously described subtypes A and B (Archer and Pomeroy, 2011; Witt et al., 2011), here named PF-EPN-A and PF-EPN-B. PF-EPN-A tumors, the largest of the ependymoma subgroups comprising nearly 50% of all cases, have a stable genome, are CIMP-positive, and have a remarkably poor outcome. PF-EPN-B has the most unstable genome of all ependymomas profiled here, with many DNA copy number gains and losses most often of whole chromosomes.

Supratentorial tumors are divided into subependymoma (ST-SE) and two subgroups characterized by molecular fusions. The largest supratentorial subgroup, ST-EPN-RELA, which has exceptionally poor prognosis, is characterized by expression of *C11orf95-RELA* fusion transcripts, shown in earlier work to occur in supratentorial ependymomas and to be sufficient for oncogenesis when expressed in stem cells (Parker et al., 2014). These fusions are hypothesized to activate NF-κB signaling. Interestingly, within this subgroup, a novel *PTEN-TAS2R1* fusion transcript was found in a *RELA* fusion negative tumor, hypothesized to activate NF-κB through loss of PTEN activity which activates the Akt/mTOR pathway.

In earlier work, *CDKN2A/B* homozygous deletion had been shown to correlate with poor prognosis of supratentorial ependymomas (Korshunov et al., 2010). Here, Pajtler et al. (2015) show that these events occur exclusively in the RELA subgroup. It is not yet clear whether the CDKN2A changes themselves drive poor outcome or whether they just correlate with the poor outcome ST-EPN-RELA group at large. The ST-EPN-RELA subgroup also has the most copy number abnormalities of tumors in this compartment, including loss of chr9/9q and chr11, and is the only subgroup with evidence of chromothripsis among the supratentorial subgroups.

Finally, *YAP1* fusions, primarily *YAP1-MAMLD1*, described previously characterize ST-EPN-YAP1, the third supratentorial subgroup. It is not clear how this fusion drives tumor growth, although MAMLD1 and MAML2, both members of the Mastermind gene family, are known co-activators of NOTCH. A novel *YAP1* fusion is described, where the c-terminus *trans*-activating domain of *YAP1* is exchanged for the *FAM118B* protein coding region. YAP1 is a member of the tumor suppressor hippo pathway that regulates cell growth, contact inhibition, apoptosis, and differentiation (Fernandez-L and Kenney, 2010). YAP1 acts downstream of SHH to regulate proliferation of granule cells during cerebellar development. It is not known how these *YAP1*-truncating fusions drive tumor growth, but YAP1 has been implicated in a variety of cancer phenotypes (Fernandez-L and Kenney, 2010).

The two most common subgroups, PF-EPN-A and ST-EPN-RELA, together comprise over 65% of all cases and, by a large margin, have the worst prognosis (Figure 1). These tumors tend to affect young patients, and they are remarkably resistant to treatment. Equally remarkable, patients in the other subgroups have an excellent prognosis—nearly all have close to 100% overall survival after 5 years, which holds true even if the tumors recur.

If validated in an independent cohort, these findings argue for a change in the current approach to risk stratification and treatment. Molecular subtyping proved to be more accurate for predicting outcome than histological grading. Multivariate analysis demonstrated molecular subgroup, degree of surgical resection, and gain of 1q as independent prognostic parameters. After optimal surgical resection, molecular subtyping should be considered in treatment decisions, and PF-EPN-A and ST-EPN-RELA especially should be targeted for the development of novel approaches given the identification of these subgroups with extremely poor prognosis despite aggressive therapy.

This comprehensive survey of the landscape of ependymomas creates new opportunities to improve clinical care by more accurate risk stratification. It also exposes many challenges for the field and raises new questions. Two molecularly defined subgroups, PF-EPN-A and ST-EPN-RELA, emerge as the principal causes of poor outcome. Although they are responsible for the majority of deaths,

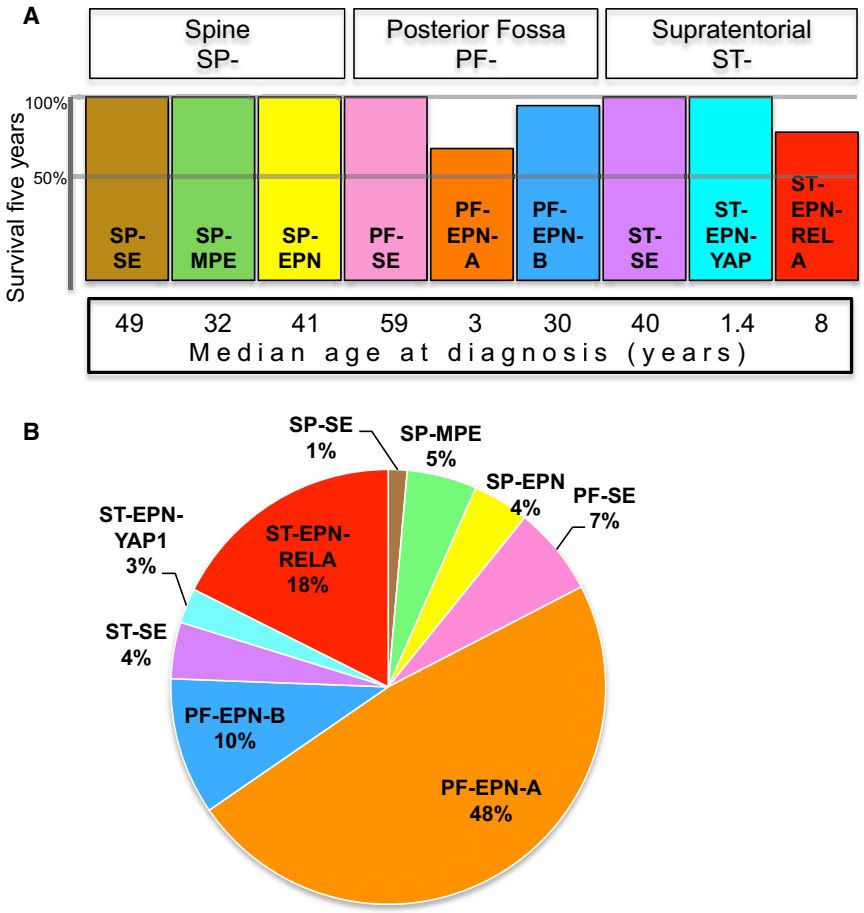

Figure 1. The Two Largest Subgroups of Ependymoma Have the Lowest Overall 5-Year Survival Rates and Are Predominantly Pediatric

(A) The overall 5-year survival rates of each subtype are listed as a bar graph. Ependymomas develop in each compartment of the CNS, spine (SP), posterior fossa (PF), and supratenorial (ST), and each compartment has three subtypes of ependymoma. Median age at diagnosis is listed for each subgroup.

(B) The frequency of patients in each subgroups of ependymoma as identified in the Pajtler et al. (2015) cohort. PF-EPN-A and ST-EPN-RELA are the most common subtypes of ependymoma.

the mechanisms of their resistance to treatment remain unclear. They share similar gene set pathway enrichments: cell cycle, cell migration, and MAPK signaling. These two subgroups also share chromosome 1q gain, which also independently predicts poor outcome. Will they respond to a similar targeted drug therapy? Faithful models of these subgroups are lacking. Molecular mechanisms of oncogenesis will need to be defined. Targeting epigenetic modifiers has been suggested as a therapeutic target because of the similar DNA methylation patterns of the worst prognosis tumors (Mack et al., 2014). There is much to do before we can begin to make any meaningful progress to improve the outcome of these treatment-resistant tumors. The tumor subgroups with good prognosis are often treated with external beam radiation, which has many long-term consequences, including neurocognitive deficits and increased risk of stroke and second cancers. All ependymoma subgroups are also resistant to conventional chemotherapy. Molecular mechanisms that can be targeted will need to be defined so that radiation can be eliminated for those who need treatment after surgery. Defining the disease landscape is a major step forward, and it highlights the challenges ahead as we work to improve the outcome of this devastating disease.

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